Potential prediction of formulation performance in paediatric patients using biopharmaceutical tools and simulation of clinically relevant administration scenarios of nifedipine and lorazepam

Running title: Biopharmaceutical tools in paediatrics

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Abstract

Aims: This study explores the impact of paediatric patient related factors and choice of formulation on the dissolution characteristics of nifedipine and lorazepam, two drug substances regularly applied in very young patients and in compounded formulations.

Methods: Dissolution experiments were designed to reflect clinical practice in a paediatric hospital, with respect to dosage forms, feeding regimens, and methods of administration. Solubility studies addressed the influence of age and prandial state. Drug solubility and dissolution experiments were conducted in biorelevant media and adapted age-specific (neonate and infant) media. Dissolution studies were performed with the mini-paddle apparatus and the flow-through cell apparatus.

Results: Dissolution of nifedipine formulations was not affected by age-related changes of the fasted state simulated gastro-intestinal fluids, and by disintegration of the formulation before administration. However, a significant difference in nifedipine's dissolution rate from commercial tablets and compounded capsules was observed. The dissolution of lorazepam tablets was affected by fasted- vs. fed-state media, but it was deemed less likely to be clinically relevant. The significant effect of fed-state media on nifedipine's solubility was considered to have possible clinical relevance since very young patients are almost continuously in a fed state.

Conclusion: The in vitro results obtained from these studies reveal the potential of biorelevant solubility and dissolution studies reflecting clinical practice to predict drug performance in paediatric patients.

Keywords

Biorelevant, dissolution, solubility, paediatric, nifedipine, lorazepam

What is already known:

- Using biorelevant dissolution, successful *in vitro in vivo* relations/correlations (IVIVCs) have been established for oral immediate and modified release formulations in adults
- Evaluation of oral drug absorption is often only performed for fasted state conditions, which are not applicable to new-borns and infants.

What this research adds:

- *In vitro* dissolution tests were conducted for two nifedipine formulations under settings considered to reflect clinical conditions. Both nifedipine conventional commercial tablets and compounded capsules displayed slow dissolution in fasted state conditions.
- Our biorelevant solubility results suggest that there is a food effect on the solubility of nifedipine in gastrointestinal fluids in neonates and infants, which might be translated into an effect on *in vivo* drug absorption after administration of conventional formulations.

1. Introduction

During the last decades, the purpose of dissolution testing of drugs has expanded from pure quality control to prediction of *in vivo* drug performance and identifying potential bioavailability problems of pharmaceutical formulations. With development of biorelevant dissolution media (reflecting the main properties of gastrointestinal fluids), simulation of gastro-intestinal residence times, and simulation of gastro-intestinal hydrodynamics, successful *in vitro in vivo* relations and correlations (IVIVRs/ IVIVCs) have been established for oral immediate and modified release formulations in adults, using compendial dissolution apparatus [1], [2]. Furthermore, *in vitro* dissolution data are used as input in physiologically based pharmacokinetic (PBPK) prediction models in which different factors influencing drug absorption are integrated. Both biorelevant dissolution and PBPK modelling are regularly applied in drug development by the pharmaceutical industry, i.e. to select and optimize formulations, or to predict solubilisation and precipitation in the human gastro-intestinal tract under various conditions [3]. Ideally, *in vitro* predictive methods, combined with *in silico* models, will replace *in vivo* experiments and clinical trials.

In recent years, advances towards the availability of suitable paediatric biorelevant dissolution tests have been made with the development of paediatric media [4]. These media were based on the available literature on the composition of paediatric luminal fluids and the established adult media [5]. Experimental dissolution parameters that mimic paediatric conditions have been proposed [6], [7]. The search for alternatives to clinical trials is essential, especially in paediatrics, given the ethical and methodological difficulties involved in performing trials in paediatric patients [8]. Predictive biopharmaceutical methods representing the *in vivo* drug dissolution in children would be of huge benefit for early formulation screening and assessing the influence of different administration strategies on drug performance, with the ultimate goal to reduce the amount of *in vivo* studies required and accelerating paediatric drug development.

Apart from the obvious differences between children and adults regarding the physiological development of the gastro-intestinal tract, the effect of pharmacotherapy in paediatric patients can be further influenced by a range of patient-related factors, such as feeding regimens, the presence of feeding tubes, immobility, and the selected drug formulation. Pharmacists often have to rely on extemporaneous compounding and unlicensed manufacturing, or manipulation of adult dosage forms, in case licensed, age-appropriate paediatric drug products are not available.

This study aims to explore the application of biopharmaceutical methods to study the impact of patient related factors on drug performance in paediatric patients, using two biopharmaceutical tools: drug substance solubility and drug product dissolution in biorelevant media adapted to reflect paediatric conditions. Dissolution experiments were designed to simulate the impact of formulation handling and dosage form manipulation. Dissolution parameters were set based on what is currently known about physiological conditions in the GI tract of children. To assess the age- and prandial state related changes in paediatric gastrointestinal solubility, respective studies were performed in both adult biorelevant media and adapted paediatric media.

The compounds that were chosen to be studied were nifedipine and lorazepam, as they are both regularly applied in unlicensed formulations and in very young patients. Nifedipine is a biopharmaceutical classification system (BCS) class II drug substance, with an aqueous solubility of around 5-9 µg/ml [9] and a logP value of 2.20 [10]. With pKa values of -0.9 and 13, it is not ionisable in the gastro-intestinal pH range. Therefore, under physiologically relevant conditions, nifedipine acts as a neutral molecule and its solubility is independent of the pH of the medium [11]. Lorazepam is a compound with a slightly better aqueous solubility (80 µg/ml) compared to nifedipine, and a logP value of 2.39 [12]. With pKa values of 1.3 and 11.5 [13], it can be partly ionised within the acidic conditions of the fasted stomach. Using the above stated

106 solubility value, a dose number (D_0) ≤ 1 is calculated for dose strengths up to 20 mg, and thus
107 lorazepam would be considered as a highly soluble, class I compound within the ‘adult’ BCS.

2. Materials and methods

2.1. Materials

Pepsin from porcine gastric mucosa (powder, ≥400 units/mg protein), nifedipine drug substance (≥98% HPLC grade), lorazepam reference standard (≥98% HPLC grade) and Whatman GF/D (pore size 2.7 μm, 25 mm diameter) and GF/F (pore size 0.7 μm, 25 mm diameter) filters were purchased from Sigma–Aldrich (Dorset, UK). UHMW polyethylene 10 micron full flow cannula filters were bought from Quality Lab Accessories LCC (Telford, USA). Egg-lecithin (Lipoid E PCS) was purchased from Lipoid GmbH (Ludwigshafen, Germany). Sodium taurocholate (NaTc) was purchased from Prodotti Chimici e Alimentari S.p.A (Basaluzzo, AL, Italy). Cronus 13 mm regenerated cellulose (RC) syringe filters 0.45 μm were purchased from LabHut Ltd (Maisemore, UK). Aptamil 1 (Nutricia, Trowbridge, UK), SMA Wysoy Soya Infant Formula (SMA Nutrition, Gatwick, UK) and Ultra Heat Treated Standardised Whole Milk 3.6% fat (Sainsbury's, London, UK) were purchased from a local supermarket. Water was of Milli-Q grade. All other reagents and chemicals were of analytical grade and were used as received, without further purification.

2.2. Instrumentation

Equipment utilized in the current study included a R114 Rotavapor (Buchi, Flawil, Switzerland), a SevenCompact pH/Ion S220 pH meter (Mettler-Toledo AG, Schwerzenbach, Switzerland), Heraeus Fresco 17 and Heraeus Biofuge Primo R centrifuges (Thermo Scientific, Hanau, Germany), an Agilent Technologies 708-DS (USP II) apparatus configured with Agilent TruAlign 200 ml vessels and Agilent electropolished stainless steel mini-paddles (Santa Clara, CA), a Sotax CE7 smart flow-through cell (USP IV) apparatus connected to a CP 7 Piston Pump (Sotax, Switzerland). The Agilent 1100 HPLC system consisted of a G1311A Quaternary Pump, G1315A DAD detector, G1316A Column Compartment, G1322A Degasser, G1329A

Autosampler, G1330A Autosampler Thermostat and ChemStation® software (Agilent Technologies, Santa Clara, US).

2.3. Drug products

Conventional commercial nifedipine "retard" 10 mg tablets (Centrafarm, Etten-Leur, the Netherlands), unlicensed GMP-grade nifedipine 1 and 5 mg capsules (Apotheek A15, Gorinchem, the Netherlands), commercial lorazepam 1 mg IR tablets (Mylan, Bunschoten, The Netherlands) and unlicensed GMP-grade lorazepam oral solution 1 mg/ml (Apotheek A15, Gorinchem, the Netherlands) were used. Nifedipine capsules were compounded from pure API into hard gelatine capsules using lactose as single excipient. Lorazepam oral solution 1 mg/ml contains glycerol *85*%v/v (87%v/v), polyethylene glycol (PEG) 400 (10%v/v) and propylene glycol (3%v/v) [14]. All formulations used in this study were part of the formulary of the Sophia Children's Hospital, Rotterdam, The Netherlands.

Although no dosing advice is available for neonates, in clinical practice nifedipine is sometimes administered to patients below the age of one month, in dosages from 0.1 up to 1.0 mg/kg [25]. For experiments simulating neonatal or infant conditions, nifedipine unlicensed 1 mg capsules were used to reflect clinical practice.

Oral lorazepam is used off-label to gradually taper-off benzodiazepines that have been administered as continuous intravenous sedation at the paediatric intensive care unit (PICU). It is usually administered 4 times daily, in single dosages up to 0.5 mg (neonates), 1 mg (infants) and 3 mg (children) [15]. To allow for precise dosing and ease of administration, a 1 mg/ml oral solution was developed specifically for paediatric patients [14].

2.4. Media used for solubility and dissolution studies

Simulated Gastric Fluid without pepsin (SGF *sp*) pH 1.2 and Simulated Intestinal Fluid without pancreatin (SIF *sp*) pH 6.8 were used in dissolution studies [16]. Freshly prepared adult and age-specific (neonate and infant) biorelevant media were used in solubility and dissolution studies (Table 1) [4], [5], [16], [17].

2.5. In vitro dissolution studies

2.5.1. Experimental set-up

Dissolution experiments were performed with the mini-paddle apparatus and the flow-through cell apparatus. The mini-paddle apparatus is particularly suitable for working with reduced fluid volumes, to better mimic intraluminal fluid volumes in the GI tract of paediatric patients [18]. The flow-through cell apparatus (USP IV apparatus) offers the advantage of easily changing the medium and flow rate during an experiment, and maintaining sink conditions when operated in the open mode [19].

The mini-paddle apparatus was equipped with 200 ml vessels and matching paddles, using a smaller volume compared to adult biorelevant studies [1]. As intestinal motor activity matures throughout early infancy [20], the agitation rate of the paddle was set at 50 rotations per minute (RPM). 2 ml samples were removed (with sample replacement) using a 5mL Fortuna Optima® syringe fitted with stainless tubing and a cannula filter to facilitate representative sampling.

The flow-through cell apparatus was equipped with large cells (22.6 mm diameter), with a 5 mm ruby bead at the bottom of the cell and small glass beads (1 mm diameter) filling the cone of the cell. Test formulations were placed on a tablet holder. On top of each cell, two filters were placed; a GF/D and a GF/F filter (Glass Microfibre Filters 24 mm, Whatman™). In all experiments, the open mode was used. Samples were collected in glass cylinders or Erlenmeyer flasks, which were weighed to determine the volume of the sample.

All experiments, both in the mini-paddle and the flow-through cell apparatus, were conducted at 37°C. Sample collection for nifedipine took place at 5, 15, 30, 40, 50, 60, 75, 90 and then every 30 minutes up to 270 minutes, after the start of the experiment. Sample collection for lorazepam took place at 5, 15, 30, 45, 60, 75, 90 and 120 minutes after the start of the experiment. Before HPLC-analysis, samples were filtered through a 0.45 µm RC filter, discarding the first 10 drops (adsorption of the drugs onto the filters was checked and confirmed to be negligible). Calibration curves were prepared in corresponding media for each experiment on the day of the experiment. All experiments were performed in triplicate and in the case of nifedipine under protection from light.

2.5.2. Screening the impact of patient related variables

To reflect clinical practice in a paediatric hospital with respect to dosage forms, feeding regimens, and methods of administration, information from standard operating procedures of the Sophia Children's Hospital was retrieved. A clinically relevant design of the *in vitro* experiments was followed by considering: i. the enteral feeding protocol, ii. the protocol for administration of medicines through a feeding tube, and iii. the local drug formulary of the Sophia Childen's Hospital. The patient relevant parameters studied in the dissolution experiments were age, prandial state, method of administration, and formulation type.

Even though oral administration is the preferred route to feed paediatric patients, enteral feeding through a nasogastric tube is often indicated, due to an inability or unwillingness of eating or swallowing, anorexia, motility problems etc. This mode of administration has implications for the gastric-emptying rate, which increases with enteral feeding compared to oral feeding [21]. When possible, breast milk is the preferred type of food for children for a minimum duration of 4-6 months from the day of birth. Otherwise, patients are fed with formula milk, adjusted to their energy and protein requirements and potential fluid restriction. Table 2 displays the

standard neonate and infant formulas administered to enterally fed patients. For normal birth-weight neonates and infants, the feeding interval is gradually reduced from 8 times a day at birth to 4 times a day at 8 months old. In certain conditions, such as gastroparesis, hyperemesis or recurrent aspiration, gastric feeding is not suitable and transpyloric feeding directly into the duodenum is required. Because the duodenum has no reservoir capacity like the stomach, transpyloric feeding is always administered as a continuous drip.

Administration of solid dosage forms to paediatric patients is often not possible, which means that the formulation has to be manipulated before administration. In the Sophia Children's Hospital, as per protocol, immediate release capsules and tablets are dispersed in an oral syringe with a small amount of lukewarm water (1-20 ml).

2.5.3. Nifedipine solubility studies

To study the influence of age-related changes in GI fluid composition on compound solubility, nifedipine solubility studies were performed according to methods described by Maharaj et al. [4]. In summary, for aqueous based media, an excess amount of nifedipine was added to 2 mL of medium, dwelled for 24 hours at a shaking water bath at 37°C, filtered through an 0.45 µm RC filter and diluted with fresh medium prior to HPLC-analysis. For the milk-based media, a drug extraction step was required, which consisted of a centrifugation step, precipitation of proteins with methanol, a second centrifugation and filtration of the resulting supernatant through an 0.45 µm RC filter. All solubility experiments were conducted in triplicate.

2.5.4. Nifedipine dissolution studies

An overview of the dissolution experiments is given in Table 3. Firstly, pH changes in the fasted state, resulting from passage through the stomach and small intestine, were simulated in the mini-paddle apparatus using SGF*sp* and SIF*sp* (see section 2.4). The pH shift from the gastric to the intestinal conditions was achieved by the addition of an equal volume of double concentrated SIF*sp* (with additional NaOH), after a simulated gastric residence time of 45 minutes. Secondly, to compare the dissolution of the two nifedipine formulations that are regularly used in the Sophia Children's Hospital, commercial nifedipine 10 mg tablets and compounded 5 mg capsules, an experiment was performed in the flow-through cell apparatus. To simulate administration to fasted children, adult biorelevant media were used (FaSSGF/FaSSIF), the gastric residence was set at 30 minutes and the flow rate was reduced from 5ml/min to 4 ml/min after the medium switch in order to reflect the in vivo gastric and intestinal conditions (in terms of residence time and volume). The dose of the 10 mg tablets was matched by using two 5 mg capsules per cell. Thirdly, a dissolution experiment in fasted state neonatal media (Pn-FaSSGF/FaSSIF) was performed with the flow-through cell apparatus, in order to reveal the effect of different hydrodynamics compared to the mini-paddle apparatus.

The effects of age-related differences in gastro-intestinal conditions on dissolution were simulated with the use of neonatal (Pn-FaSSGF/FaSSIF) and infant (Pi-FaSSGF/FaSSIF) fasting media in the mini-paddle apparatus (see Table 3). To reflect the *in vivo* conditions in neonates, the gastric residence time was prolonged, and the gastric volume was decreased compared to infants [8]. Thirdly, administration through a gastric feeding tube, where the capsule is dispersed in an oral syringe with 5 ml of warm water before administration, was also simulated in fasted state neonatal media (Pn-FaSSGF/FaSSIF).

2.5.5. Lorazepam dissolution studies

Details of the design of the dissolution studies are presented in Table 3. Dissolution of the 1 mg tablets was studied in SGF*sp* and SIF*sp* and the mini-paddle apparatus, with an increased rotational speed of 75 rpm to prevent coning of the tablet formulation. This experiment was repeated using the lorazepam 1 mg/ml oral solution to compare the profiles.

Subsequently, two experiments were conducted with the flow-through cell apparatus. The effect of prandial state on dissolution was explored using lorazepam 1 mg tablets and infant fasted state and fed state simulated gastric and intestinal fluids. Pnc-FeSSGF was considered appropriate to simulate infant fed-state gastric fluid, as it contains milk formula that is given to infants up to the age of six months. Gastric residence time in the fed state was prolonged compared to the fasted state and the flow rates were set to reflect the prandial state and gastro-intestinal tract compartment. Administration directly into the duodenum was simulated using lorazepam 1 mg tablets and infant fed state intestinal fluid (Pi-FeSSIF).

2.6. Analytical quantification

For the quantitative analysis of nifedipine, high performance liquid chromatography combined with UV (HPLC-UV) detection was used. The method was adapted from the method previously reported by Vertzoni *et al*. [22], [23]. Nifedipine was separated on an analytical C_{18} column (Thermo Hypersil GOLD, 5 μm, 250 × 4.6 mm) with UV detection at 238 nm, a column temperature of 30°C, mobile phase of a 60:40 mixture (v/v) of methanol and water (Milli-Q), a flow rate of 1.0 ml/min, and injection volume of 50 μl. For the quantitative analysis of lorazepam, the HPLC-UV method as reported by Share *et al.* was used [24]. Lorazepam was separated using a Zorbax SB-C18 analytical column (3.5 μm, 150 × 4.6 mm) with UV detection at 230 nm, a column temperature of 30°C, mobile phase of a 60:40 mixture (v/v) of methanol and water (Milli-Q), a flow rate of 0.75 ml/min, and injection volume of 20 μl. Quantification

of nifedipine and lorazepam was made based on calibration curves constructed from stock solutions in the corresponding medium (range 0.5-12 μg/ml). For milk- and formula-based media, calibration curves were created in triplicate, and the same protein precipitation, centrifugation and filtration process was applied as described in section 2.5.

2.7. Statistical analysis

One-way analysis of variance (ANOVA) with a post-hoc Tukey's test was applied to identify statistically significant differences in solubility between adult and age-specific media, using a significance level of $p \leq 0.05$. Statistical analysis was performed in GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA).

3. Results and Discussion

3.1. Paediatric gastrointestinal solubility of nifedipine

Nifedipine is a substance with poor aqueous solubility (around 5-9 µg/ml) [9]. As shown in Figure 1, nifedipine solubility in adult FaSSGF (3.81 µg/ml) was different to solubility in Pi-FaSSGF (7.04 µg/ml), but not to Pn-FaSSGF (4.99 µg/ml). Although the relativedifference between the solubility values for adult FaSSGF and Pi-FaSSGF was large, all values were very similar to the range reported for aqueous solubility. This implies that the small amounts of bile salts and pepsin present in FaSSGF have a negligible effect on nifedipine solubility, and that age-related changes in fasted state gastric fluid may be unlikely to significantly influence the absorption of nifedipine.

Compared to FaSSGF, solubility in FaSSIF was increased. Paediatric investigations examining luminal fluids within the fasted-state proximal intestine are thus far limited, therefore the different FaSSIF media were developed to explore the impact of variations in bile salt concentrations [4]. The nifedipine solubility values that were found in FaSSIF-V2 (12.9 µg/ml), FaSSIF-50% (9.3 µg/ml) an FaSSIF-150% (15.1 µg/ml) reflected these variations.

In FeSSGF, nifedipine solubility was markedly increased compared to FaSSGF, indicating a substantial effect of prandial state on nifedipine solubilisation in the gastric fluid. Additional changes in solubilisation can be expected for energy and/or protein enriched nutrition [25].

The biggest relative differences in solubility between adult and paediatric media were observed in FeSSIF. Nifedipine solubility in adult FeSSIF-V2 (45.2 µg/ml) was higher than Pi-FeSSIF (32.1 µg/ml), Pnc-FeSSIF (18.3 µg/ml) and Pnb-FeSSIF 18.5 µg/ml), reflecting the solubilizing effects of lipids and bile salts.

It should be noted that the solubility of a drug substance in a certain medium is a compound specific property. Nevertheless, the increased solubility in fed-state media could have some

implications for clinical practice. Both nifedipine formulations do not have any attributes that slow or alter the release of the drug substance. The slow dissolution rate of the drug substance itself is considered to provide the slow onset of action of the drug. In paediatric hypertension, nifedipine is administered to patients from the age of one month, who are effectively in an almost continuous fed prandial state. This means that, in these patients, a much more rapid dissolution from the nifedipine conventional tablets and capsules could be expected, possibly leading to a shortened Tmax, an increased Cmax and an altered drug exposure , compared to administration to fasted state patients. An increased Cmax may lead to typical dihydropyridine adverse effects like headache and flushing. More serious adverse events reported in paediatric patients possibly caused by nifedipine included change in neurological status, severe hypotension, and oxygen desaturation [26]. For this reason, the use of immediate release capsules, filled with a liquid solution of nifedipine, is not recommended in the Netherlands [27].

3.2. Dissolution studies

3.2.1. Nifedipine

The nifedipine dissolution results in both the mini-paddle apparatus and the flow-through cell apparatus are presented in Figure 2 and Figure 3.

3.2.2. Experimental aspects

Impact of pH - Figure 2A shows the dissolution profile of a 5 mg compounded capsule in SGF *sp* and SIF *sp* in the mini-paddle apparatus. As nifedipine is unionisable in the gastro-intestinal pH range, no apparent effect on nifedipine's dissolution from the pH switch was observed. A plateau was reached after around 180 minutes, with just over 20% of nifedipine dissolved. After the media switch, a rise in the amount dissolved was observed, resulting from an increased volume of dissolution medium. The large variability in the early phase of the experiment was caused by a variable capsule rupture time.

Impact of formulation - The dissolution profiles of the commercial 10 mg nifedipine tablets and the compounded nifedipine 5 mg capsules in FaSSGF/FaSSIF-V2 (simulating children) in the flow-through cell apparatus (displayed in Figure 2B) show a similar dissolution/release pattern, but the dissolution extent and rate from the capsules were slightly higher. As mentioned before, both formulations are presented as slow release formulations, even though they are not formulated as such. The dissolution profiles do confirm this assumption under fasted conditions, however, from our results, the capsules and tablets cannot be considered interchangeable, as they previously were [28].

Impact of hydrodynamics - Figure 2C shows the dissolution profiles of nifedipine 1 mg compounded capsules in Pi-FaSSGF/FaSSIF in the mini-paddle and the flow-through cell apparatus. The extent of nifedipine dissolution was affected by the type of *in vitro* dissolution apparatus used. Dissolution of nifedipine 1 mg capsules in the mini-paddle apparatus reached a plateau of 40% after around two hours, due to the lack of sink conditions, whereas in the flow-through cell apparatus a continuous dissolution of nifedipine was observed with 70% dissolved at 270 min. Due to the continuous flow of fresh medium, sink conditions were achieved when the system operated in the open-loop configuration. In this way, the dissolution rate reflects the behaviour of the formulation and not the solubility of the substance, as in the closed systems [19].

3.2.3. Patient related aspects

Impact of age - Figure 3A shows the dissolution profiles of 1 mg compounded capsules in Pn-FaSSGF/FaSSIF-V2 and Pi-FaSSGF/FaSSIF-V2 in the mini-paddle apparatus. Between the dissolution profiles, a small effect of age was observed due to the difference in gastric emptying time/media switch, but an overall similar extent of dissolution was seen at the end of the experiment. This was an expected result as dissolution conditions with regard to fluid composition only moderately differed in the gastric phase. The gastric release was again

variable and low in both experiments, as a result of a variable capsule rupture time. Also, the bile salt content, an important constituent to nifedipine solubility, is much lower in (paediatric) FaSSGF compared to FaSSIF-V2 [4].

Impact of method of administration - Figure 3B shows the dissolution profiles of nifedipine compounded capsules administered intact vs. capsule content dispersed in water before administration in Pn-FaSSGF (45 min) and FaSSIF-V2 in the mini-paddle apparatus. The amount released was slightly higher in the gastric phase in the case that the capsule content had been mixed with water before administration compared to the direct administration of the capsule, and profiles in the intestinal phase were similar (Figure 2B). Since absorption mainly takes place from the small intestine and onwards, this suggests that a large change in the rate and extent of absorption from the different mode of administration of the capsule is unlikely [29].

3.3. Lorazepam

The lorazepam dissolution results under paediatric biorelevant conditions in both the mini-paddle apparatus and the flow-through cell apparatus are presented in Figure 4.

3.3.1. Experimental aspects

Impact of pH and formulation - Performance of the lorazepam oral solution and tablet formulation was assessed in SGF *sp* and SIF *sp* with the mini-paddle apparatus (Figure 4A). During the simulated gastric residence time (45 min), almost all lorazepam was dissolved from the tablet, reaching similar concentrations as compared to the lorazepam liquid after only 15 minutes.

3.3.2. Patient related aspects

Impact of prandial state - Results from the flow-through cell apparatus dissolution studies depicted in Figure 4B show the dissolution profiles of lorazepam 1 mg tablets in Pi-FaSSGF/FaSSIF-V2 and Pnc-FeSSGF/Pi-FeSSIF. A slower dissolution rate was observed in fasted state conditions, leading to a lower amount dissolved after two hours (81.3% +/- 8.4%) compared to fed state conditions (95.3% +/-3.0%). This could be predictive of a slower rate of *in vivo* drug absorption. Nevertheless, this is a relatively small difference, considering that lorazepam is likely to be absorbed almost completely, as is known from adult data [30]..

Impact of method of administration - Sometimes a patient does not tolerate gastric feeding and transpyloric feeding directly into the duodenum is required. When necessary, medication is also administered through the duodenal feeding tube and solid dosage forms are crushed or dispersed. When direct administration of the lorazepam tablet to the duodenum was simulated in Pi-FeSSIF in the flow-through cell apparatus, the dissolution profile was similar to the one obtained in Pnc-FeSSGF/Pi-FeSSIF (Figure 4C). These results suggest that administration through a duodenal feeding tube will not impact the in vivo dissolution. A change in t_{max} is still possible however, as lorazepam reaches the site of absorption, namely the upper intestine, more quickly than when it is administered orally or via a gastric feeding tube.

4. General discussion

This study has yielded information on the application of nifedipine and lorazepam products in paediatric pharmacotherapy based on biopharmaceutical *in vitro* investigations.

As mentioned in section 3.1, markedly increased or accelerated absorption of nifedipine could lead to adverse effects such as severe hypotension, and must be avoided. In the solubility studies, we observed a significant food effect on the solubility of nifedipine in paediatric media, which is in agreement with previously reported clinical data in adults [32]. This expected impact of food is also applicable in paediatric patients, and needs to be taken into account when administering nifedipine to paediatric patients. Clinicians should be aware that they cannot rely on the slow onset of action associated with nifedipine when conventional capsules or tablets are administered to patients in the fed state. Reassuring results came from the experiment dispersing the nifedipine capsule in water before administration, a commonly applied administration technique, revealing no significant differences in dissolution and thus implying no altered bioavailability in comparison to administration of the intact capsule.

In our experiments, dissolution of the lorazepam tablets was affected to a small extend by the different experimental set-ups, namely the simulated prandial state and administration site. As there are no indications that lorazepam is a substrate to gastro-intestinal drug transporters, and absorption is almost complete in adults [30], it is also unlikely that excipients from the oral liquid will alter the lorazepam absorption compared to the tablets. When administering lorazepam to PICU patients to prevent iatrogenic withdrawal syndrome, precise dosing is required [15]. For this reason, and for ease of administration through a feeding tube, a liquid formulation would be the dosage form of choice. The fast and complete dissolution of the tablets gives reassurance about the interchangeability of liquid versus immediate release lorazepam

tablets, and are supplemented with negligible effects of prandial state and administration site on the *in vitro* performance of the lorazepam formulations.

Drug substance solubility, together with the highest dose, upper GI lumen fluid volume, and volume used for administration are key factors defining the solubility classification of a drug within the BCS. The adult BCS is widely used in support of waivers of *in vivo* bioequivalence studies for immediate release solid oral formulations containing BCS class I and III (high solubility) substances, but it has been recognised that BCS classifications do not necessarily translate to paediatric populations [33]. When the dose number ($D_{0)}$ of nifedipine is calculated using the age adjusted initial gastric volumes (V_0) proposed by Shawahna [34] and the saturated solubility values (C_s) measured for Pnc-FeSSGF, dosages up to 0.22 mg/kg would result in a $D_0 < 1$, and thus be considered BCS I. This example illustrates one of the challenges of developing a relevant paediatric BCS.

Ideally, the results obtained from *in vitro* dissolution experiments would be integrated into more complex *in silico* prediction models, which are able to include other factors influencing absorption, like gastric emptying. This physiologically based pharmacokinetic (PBPK) modeling and simulation is already commonly used in formulation development/bridging for adult medicines and provides a promising tool for paediatric *in vivo* drug performance prediction, provided we gain a better understanding of the developmental changes of the gastrointestinal tract in the paediatric population [3]. Aside from the factors influencing *in vivo* dissolution, specific research is still required on the factors influencing permeability, mainly the ontogeny of metabolizing enzymes and drug transporters, to better predict oral drug absorption in this population [35]. Ultimately, the developed biopharmaceutical tools could be validated using paediatric pharmacokinetic data, when available and possible to be shared by the pharmaceutical industry. The validated biopharmaceutical tools can then be used to study off-patent paediatric drugs that would otherwise be neglected.

There is still a knowledge gap concerning GI physiology in paediatric patients. With the development of the paediatric biorelevant media, extrapolations from adult values had to be made for some aspects when availability of paediatric data was limited [4]. Future adaptations of the media compositions are therefore likely, when clinical investigations yield more accurate paediatric values. Other aspects that would gain from future clinical information and would be further updated in the design of *in vivo* predictive dissolution tests for the paediatric population would relate to the fluid volumes available at the gastro-intestinal lumen, and the motility patterns and hydrodynamics.

5. Conclusion

The *in vitro* results obtained from the experiments in this study, designed to reflect clinical practice in a paediatric hospital, suggest that biorelevant solubility and dissolution studies could assist in the understanding of drug performance in paediatric patients. The dissolution setups aiming to simulate physiological conditions to address numerous different administration scenarios, which would not be feasible or ethical in pharmacokinetic studies in children.

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455 References

456 1. Mercuri A, Fares R, Bresciani M, Fotaki N. An in vitro-in vivo correlation study for nifedipine
457 immediate release capsules administered with water, alcoholic and non-alcoholic beverages: Impact
458 of in vitro dissolution media and hydrodynamics. Int J Pharm. 2016;499(1-2):330-42.
459 2. Andreas CJ, Tomaszewska I, Muenster U, van der Mey D, Mueck W, Dressman JB. Can dosage
460 form-dependent food effects be predicted using biorelevant dissolution tests? Case example
461 extended release nifedipine. Eur J Pharm Biopharm. 2016;105:193-202.
462 3. Lennernas H, Lindahl A, Van Peer A, Ollier C, Flanagan T, Lionberger R, et al. In Vivo Predictive
463 Dissolution (IPD) and Biopharmaceutical Modeling and Simulation: Future Use of Modern
464 Approaches and Methodologies in a Regulatory Context. Mol Pharm. 2017;14(4):1307-14.
465 4. Maharaj AR, Edginton AN, Fotaki N. Assessment of Age-Related Changes in Pediatric
466 Gastrointestinal Solubility. Pharm Res. 2016;33(1):52-71.
467 5. Jantratid E, Janssen N, Reppas C, Dressman JB. Dissolution media simulating conditions in the
468 proximal human gastrointestinal tract: an update. Pharm Res. 2008;25(7):1663-76.
469 6. Karkossa F, Krueger A, Urbaniak J, Klein S. Simulating Different Dosing Scenarios for a Child-
470 Appropriate Valproate ER Formulation in a New Pediatric Two-Stage Dissolution Model. AAPS
471 PharmSciTech. 2017;18(2):309-16.
472 7. Brassine C, Fotaki N. Age related biorelevant dissolution testing for paediatric pellet
473 formulations. AAPS annual meeting; San Antonio2013.
474 8. Batchelor HK, Fotaki N, Klein S. Paediatric oral biopharmaceutics: key considerations and
475 current challenges. Advanced drug delivery reviews. 2014;73:102-26.
476 9. Gajendran J, Kramer J, Shah VP, Langguth P, Polli J, Mehta M, et al. Biowaiver Monographs
477 for Immediate-Release Solid Oral Dosage Forms: Nifedipine. J Pharm Sci. 2015;104(10):3289-98.
478 10. Masumoto K, Takeyasu A, Oizumi K, Kobayashi T. [Studies of novel 1,4-dihydropyridine Ca
479 antagonist CS-905. I. Measurement of partition coefficient (log P) by high performance liquid
480 chromatography (HPLC)]. Yakugaku Zasshi. 1995;115(3):213-20.
481 11. Mannhold R, Rodenkirchen R, Bayer R, Haas W. The importance of drug ionization for the
482 action of calcium-antagonists and related compounds. Arzneimittelforschung. 1984;34(4):407-9.
483 12. Hansch CH, Leo AJ, Hoekman D. Exploring QSAR. Dl. [2], Dl. [2]. Washington, D.C.: American
484 Chemical Society; 1995.
485 13. O'Neil MJ. The Merck index : an encyclopedia of chemicals, drugs, and biologicals.
486 Whitehouse Station, N.J.: Merck; 2006.
487 14. van der Vossen AC, van der Velde I, Smeets OS, Postma DJ, Eckhardt M, Vermes A, et al.
488 Formulating a poorly water soluble drug into an oral solution suitable for paediatric patients;
489 lorazepam as a model drug. Eur J Pharm Sci. 2017;100:205-10.
490 15. van der Vossen AC, van Nuland M, Ista EG, de Wildt SN, Hanff LM. Oral lorazepam can be
491 substituted for intravenous midazolam when weaning paediatric intensive care patients off sedation.
492 Acta Paediatr. 2018.
493 16. Galia E, Nicolaides E, Horter D, Lobenberg R, Reppas C, Dressman JB. Evaluation of various
494 dissolution media for predicting in vivo performance of class I and II drugs. Pharm Res.
495 1998;15(5):698-705.
496 17. Vertzoni M, Dressman J, Butler J, Hempenstall J, Reppas C. Simulation of fasting gastric
497 conditions and its importance for the in vivo dissolution of lipophilic compounds. Eur J Pharm
498 Biopharm. 2005;60(3):413-7.
499 18. Klein S, Shah VP. A standardized mini paddle apparatus as an alternative to the standard
500 paddle. AAPS PharmSciTech. 2008;9(4):1179-84.
501 19. Fotaki N. Flow-Through Cell Apparatus (USP Apparatus 4): Operation and Features.
502 Dissolution Technol Dissolution Technologies. 2011;18(4):46-9.
503 20. Berseth CL. Gestational evolution of small intestine motility in preterm and term infants. The
504 Journal of Pediatrics. 1989;115(4):646-51.

21. Chen W, Codreanu I, Yang J, Li G, Servaes S, Zhuang H. Tube feeding increases the gastric-emptying rate determined by gastroesophageal scintigraphy. Clin Nucl Med. 2013;38(12):962-5.
22. Vertzoni MV, Reppas C, Archontaki HA. Sensitive and simple liquid chromatographic method with ultraviolet detection for the determination of nifedipine in canine plasma. Analytica Chimica Acta. 2006;573–574:298-304.
23. Baxevanis F, Kuiper J, Fotaki N. Strategic drug analysis in fed-state gastric biorelevant media based on drug physicochemical properties. Eur J Pharm Biopharm. 2018.
24. Share MJ, Harrison RD, Folstad J, Fleming RA. Stability of lorazepam 1 and 2 mg/mL in glass bottles and polypropylene syringes. Am J Health Syst Pharm. 1998;55(19):2013-5.
25. Martir J, Flanagan T, Mann J, Fotaki N. Impact of co-administration of food and drink vehicles on the solubility of poorly soluble paediatric drugs. AAPS annual meeting; San Diego AAPS published abstracts; 2017.
26. Egger DW, Deming DD, Hamada N, Perkin RM, Sahney S. Evaluation of the safety of short-acting nifedipine in children with hypertension. Pediatr Nephrol. 2002;17(1):35-40.
27. Stichting Nederlands Kenniscentrum voor Farmacotherapie bij Kinderen. Monografie Nifedipine 2017 [Available from: https://www.kinderformularium.nl/geneesmiddel/417/nifedipine.
28. Dressman JB, Reppas C. In vitro-in vivo correlations for lipophilic, poorly water-soluble drugs. Eur J Pharm Sci. 2000;11 Suppl 2:S73-80.
29. Bode H, Brendel E, Ahr G, Fuhr U, Harder S, Staib AH. Investigation of nifedipine absorption in different regions of the human gastrointestinal (GI) tract after simultaneous administration of 13C- and 12C-nifedipine. Eur J Clin Pharmacol. 1996;50(3):195-201.
30. Greenblatt DJ, Shader RI, Franke K, MacLaughlin DS, Harmatz JS, Allen MD, et al. Pharmacokinetics and bioavailability of intravenous, intramuscular, and oral lorazepam in humans. J Pharm Sci. 1979;68(1):57-63.
31. Gonzalez D, Chamberlain JM, Guptill JT, Cohen-Wolkowiez M, Harper B, Zhao J, et al. Population Pharmacokinetics and Exploratory Pharmacodynamics of Lorazepam in Pediatric Status Epilepticus. Clin Pharmacokinet. 2017;56(8):941-51.
32. Ilic M, Kovacevic I, Parojcic J. Deciphering nifedipine in vivo delivery from modified release dosage forms: Identification of food effect. Acta Pharm. 2015;65(4):427-41.
33. Batchelor H, Ernest T, Flanagan T, Klein S, Turner R, Fotaki N, et al. Towards the development of a paediatric biopharmaceutics classification system: Results of a survey of experts. Int J Pharm. 2016;511(2):1151-7.
34. Shawahna R. Pediatric Biopharmaceutical Classification System: Using Age-Appropriate Initial Gastric Volume. Aaps j. 2016;18(3):728-36.
35. Mooij MG, de Koning BAE, Huijsman ML, de Wildt SN. Ontogeny of oral drug absorption processes in children. Expert Opinion on Drug Metabolism & Toxicology. 2012;8(10):1293-303.

543 *Table 1 Adult and paediatric biorelevant media used in solubility and dissolution experiments*
544 *[4], [5], [16], [17]*

	Fasted-state Simulated Gastric Fluid			Fed-state Simulated Gastric Fluid			
	Adult (FaSSGF)	Neonate (Pn-FaSSGF)	Infant (Pi-FaSSGF)	Adult (FeSSGF)	Neonate - Cow Formula (Pnc-FeSSGF)	Neonate - Soy Formula (Pns-FeSSGF)	
Sodium Chloride (mM)	34.2	34.2	34.2	237.02	100.35	94.79	
Sodium Taurocholate (uM)	80	20	60				
Lecithin (uM)	20	5	15				
Pepsin (mg/mL)	0.1	0.015	0.025				
Acetic Acid (mM)				17.12	7.25	7.25	
Sodium Acetate (mM)				29.75	64.65	64.65	
Milk:buffer				1:1	1:1	1:1	
HCl/NaOH qs	pH 1.6	pH 1.6	pH 1.6	pH 5	pH 5.7	pH 5.7	
pH	1.6	1.6	1.6	5	5.7	5.7	
Osmolarity (mOsm/kg)	120.7 +/- 2.5	120.7 +/- 2.5	120.7 +/- 2.5	400	340	240	
Buffering Capacity (mmol/L/ ΔpH)	-	-	-	25	15	15	
	Fasted-state Simulated Intestinal Fluid			Fed-state Simulated Intestinal Fluid			
	Adult (FaSSIF-V2)	FaSSIF-50%	FaSSIF-150%	Adult (FeSSIF-V2)	Neonate - Breast Fed (Pnb-FeSSIF)	Neonate - Cow Formula (Pnc-FeSSIF)	Infant - Cow Formula (Pi-Fessif)
Sodium hydroxide (mM)	34.8	34.8	34.8	81.65	81.65	81.65	81.65
Sodium Taurocholate (mM)	3	1.5	4.5	10	2.5	2.5	7.5
Lecithin (mM)	0.2	0.1	0.3	2	0.5	0.5	1.5

Sodium Chloride (mM)	68.62	68.62	68.62	125.5	95	111.73	107.35
Maleic acid (mM)	19.12	19.12	19.12	55.02	55.02	55.02	55.02
Glyceryl monooleate (mM)				5	5	6.65	5
Sodium monooleate (mM)				0.8	0.8	1.06	0.8
HCl/NaOH qs	pH 6.5	pH 6.5	pH 6.5	pH 5.8	pH 5.8	pH 5.8	pH 5.8
Osmolarity (mOsm/kg)	180 +/- 10	180 +/- 10	180 +/- 10	300 +/- 10	330 +/- 10	330 +/- 10	390 +/- 10
Buffering Capacity (mmol/L/ ΔpH)	10	10	10	25	25	25	25

P=paediatric, i = infant, n = neonate, c = cow milk formula, s = soy milk formula, b = breast fed

Table 2 Neonate and infant nutrition

				PICU non ventilated		PICU ventilated	
Weight (kg)	Age (months)	Standard	Caloric content per 100 ml	Standard + energy enriched	Caloric content per 100 ml	Standard + energy and protein enriched	Caloric content per 100 ml
2-3.5	0-1	Nenatal Start	78 kcal 2.5 g protein	Nenatal Start 18% w/v	87 kcal 2.7 g protein	Nenatal Start 18% w/v + 0.5% NPF	89 kcal 3.2 g eiwit
3.5-8	0-6	Nutrilon® 1	66 kcal 1.3 g protein	Nutrilon® 1 17% w/v	82 kcal 1.6 g protein	Infatrini®	100 kcal 2.6 g protein
8 - 9.5	7-9	Nutrilon® 2	68 kcal 1.4 g protein	Nutrilon® 2 17% w/v	79 kcal 1.6 g protein	Infatrini®	100 kcal 2.6 g protein
9.5 – 10.5	10-12	Nutrilon® 3	70 Kcal 1,5 g protein	Nutrilon® 3 17% w/v	79 kcal 1.7 g protein	Infatrini®	100 kcal 2.6 g protein

Nutrilon® = Aptamil® first milk, NPF= Nutrilon® Nenatal Protein Fortifier, PICU = paediatric intensive care unit

All products are manufactured by Danone (Paris, France)

552 *Table 3 Parameters used for the dissolution experiments in the mini-paddle and the flow-through-cell apparatus (USP IV apparatus).*

				Gastric Conditions				Intestinal conditions				
Apparatus	API	Formulation	Agitation (rpm)	Medium	pH	Volume (ml)	Time (min.)	Medium	pH	Volume (ml)	Time (min.)	Total Volume (ml)
Mini-paddle	Nifedipine	Capsule 5 mg	50	SGF*sp*	1.2	100	45	SIF *sp*	6.8	100	225	200
		Capsule 1 mg	50	Pn-FaSSGF	1.6	50	45	FaSSIF	6.5	150	225	200
		Capsule 1 mg	50	Pi-FaSSGF	1.6	75	30	FaSSIF	6.5	125	240	200
		Capsule 1 mg, dissolved in syringe	50	Pn-FaSSGF	1.6	50	45	FaSSIF	6.5	150	225	200
	Lorazepam	Oral solution 1 ml (1 mg/ml)	75	SGF*sp*	1.2	100	45	SIF *sp*	6.8	100	75	200
		Tablet 1mg	75	SGF*sp*	1.2	100	45	SIF *sp*	6.8	100	75	200
				Medium	pH	Flow (ml/min)	Time (min.)	Medium	pH	Flow (ml/min)	Time (min.)	Total Volume (ml)
Flow-through cell	Nifedipine	Capsule 1mg		Pi-FaSSGF	1.6	4	30	FaSSIF	6.5	3	240	840
		Capsule 2x5 mg		FaSSGF	1.6	5	30	FaSSIF	6.5	4	240	1110
		Tablets retard 10 mg		FaSSGF	1.6	5	30	FaSSIF	6.5	4	240	1110

Lorazepa								6.		
m	Tablet 1mg	Pi-FaSSGF	1.6	4	30	FaSSIF	4	3	90	390
						Pi-	5.			
	Tablet 1mg	Pnc-FeSSGF	5.7	5	60	FeSSIF	8	5	60	600
						Pi-	5.			
	Tablet 1mg					FeSSIF	8	5	120	600

API = active pharmaceutical ingredient, rpm = rotations per minute

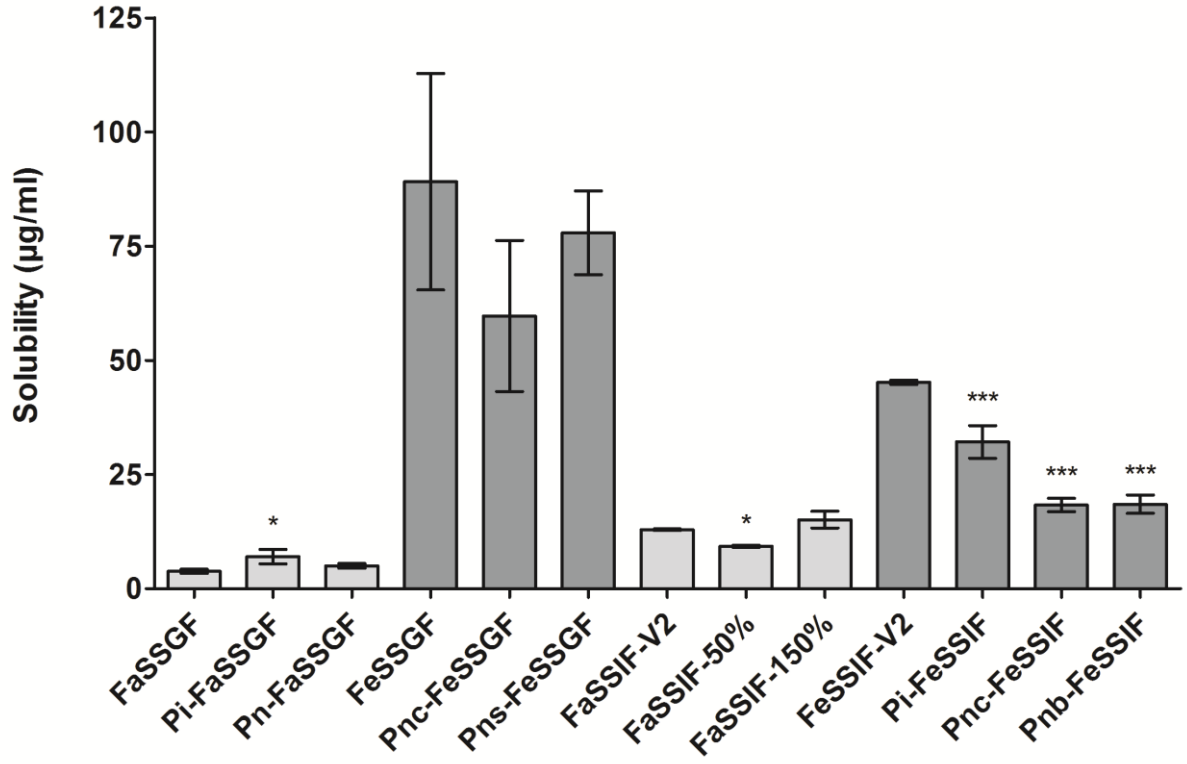
Figure 1 Nifedipine 24h solubility (mean±SD, n=3) in adult and paediatric biorelevant gastrointestinal media. Statistically significant solubility differences compared to the adult media are denoted with * ($p \leq 0.05$) or *** ($p \leq 0.001$). p=paediatric, i = infant, n = neonate, c = cow milk formula, s = soy milk formula, b = breast fed

Figure 2 Dissolution profiles of nifedipine (mean±SD, n=3) under different conditions A: nifedipine 5 mg compounded capsules in SGF sp (45min)/SIF sp (225 min), mini-paddle apparatus (50 rpm), B: nifedipine 10 mg commercial tablets (black circels) vs. 2x5mg compounded capsules (grey squares) in FaSSGF (30 min, 5 ml/min)/FaSSIF (240 min, 4 ml/min), flow-through cell apparatus, C: nifedipine 1 mg compouded capsules in Pi-FaSSGF (30 min)/FaSSIF(240 min) in the mini-paddle apparatus (50 rpm) and the flow-through cell apparatus (4 ml/min and 3 ml/min) (mean±SD, n=3). Dotted lines represent the time of medium change.

Figure 3 Dissolution profiles of nifedipine (mean±SD, n=3) under different conditions A: nifedipine 1 mg compounded capsules in Pn-FaSSGF (45 min)/ FaSSIF-V2 (225 min) (black circels) and Pi-FaSSGF (30 min)/FaSSIF-V2 (240 min) (grey squares), mini-paddle apparatus (50 rpm), B: nifedipine 1 mg compounded capsules administered intact (black circels) vs. capsule content dispersed in water before administration (grey squares), in Pn-FaSSGF (45 min)/FaSSIF-V2 (225 min), mini-paddle apparatus (50 rpm). Dotted lines represent the time of medium change.

Figure 4 Dissolution profiles of lorazepam (mean±SD, n=3) under different conditions. A: 1 mg commercial tablet (black circles) vs. 1 ml compounded oral solution 1 mg/ml (grey squares), SGF sp (45 min)/SIF sp (75 min), mini-paddle apparatus (75rpm) B: 1 mg commercial tablets, fasted-state Pi-FaSSGF (30 min, 4 ml/min)/FaSSIF-V2 (90 min, 3 ml/min) vs. fed-state Pn-FeSSGF (60 min, 5 ml/min)/Pi-FeSSIF (60 min, 5 ml/min), flow-through cell apparatus, C: 1

mg commercial tablets, regular administration Pn-FeSSGF (60 min, 5 ml/min)/Pi-FeSSIF (60 min, 5 ml/min) vs. duodenal administration Pi-FeSSIF (120 min, 5 ml/min), flow-through cell apparatus. Dotted lines represent the time of medium change.


Solubility (µg/ml)
125
100
75
50
25
0
*
*
***
***
***
FaSSGF
Pi-FaSSGF
Pn-FaSSGF
FeSSGF
Pnc-FeSSGF
Pns-FeSSGF
FaSSIF-V2
FaSSIF-50%
FaSSIF-150%
FeSSIF-V2
Pi-FeSSIF
Pnc-FeSSIF
Pnb-FeSSIF

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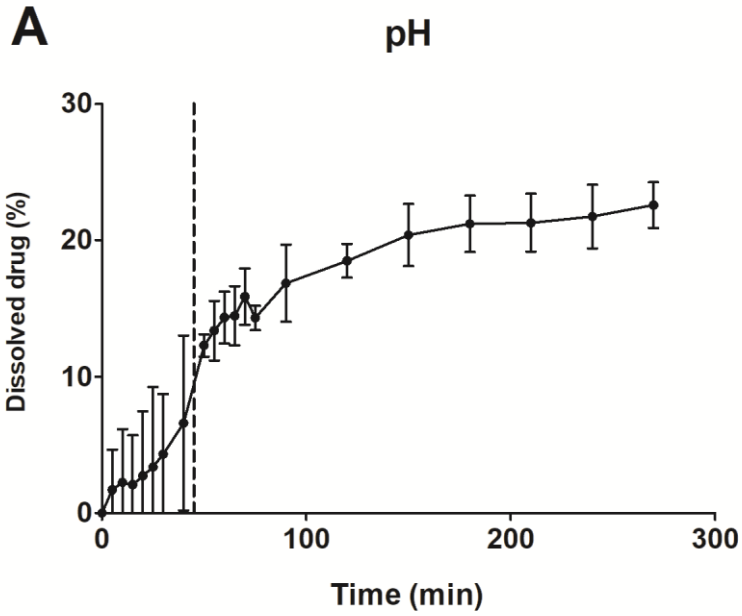
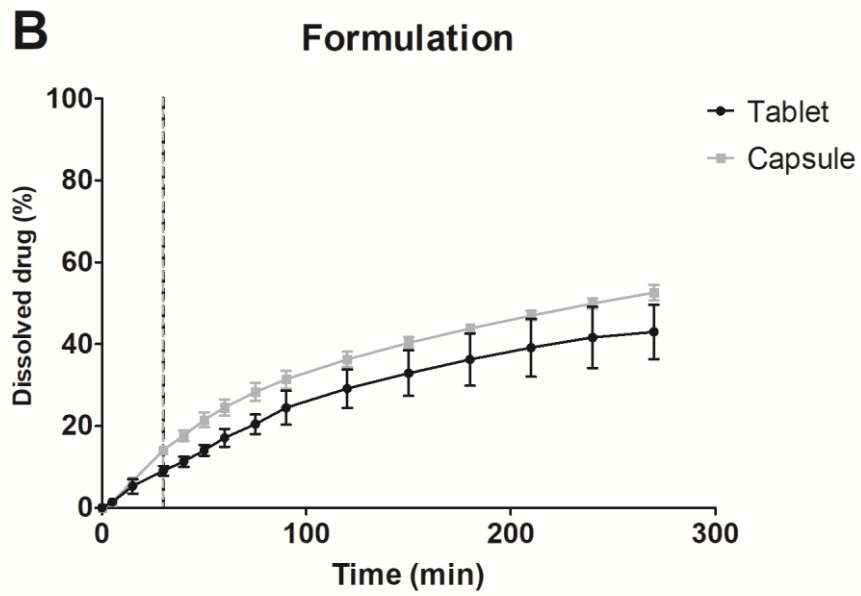
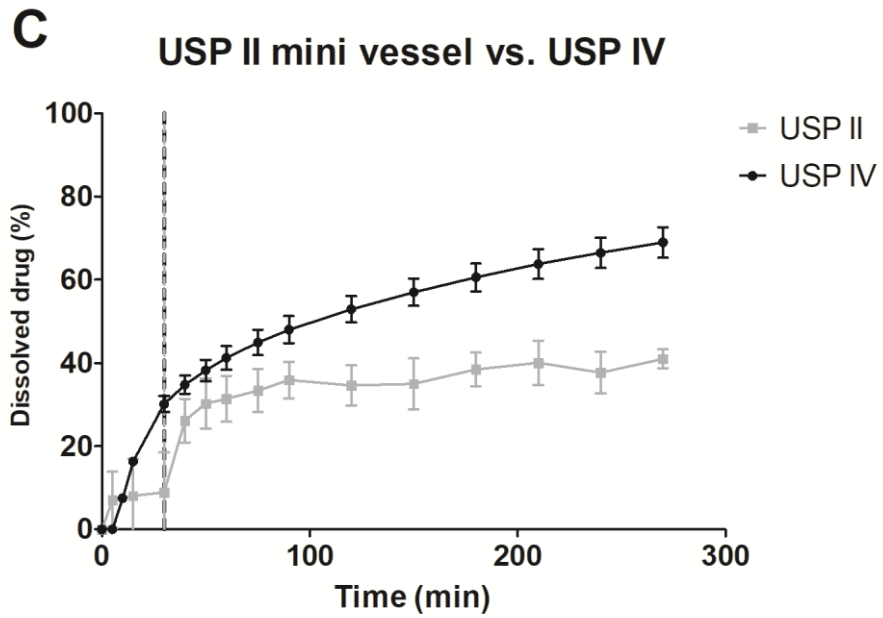

A
pH
Dissolved drug (%)
30
20
10
0
0
100
200
300
Time (min)

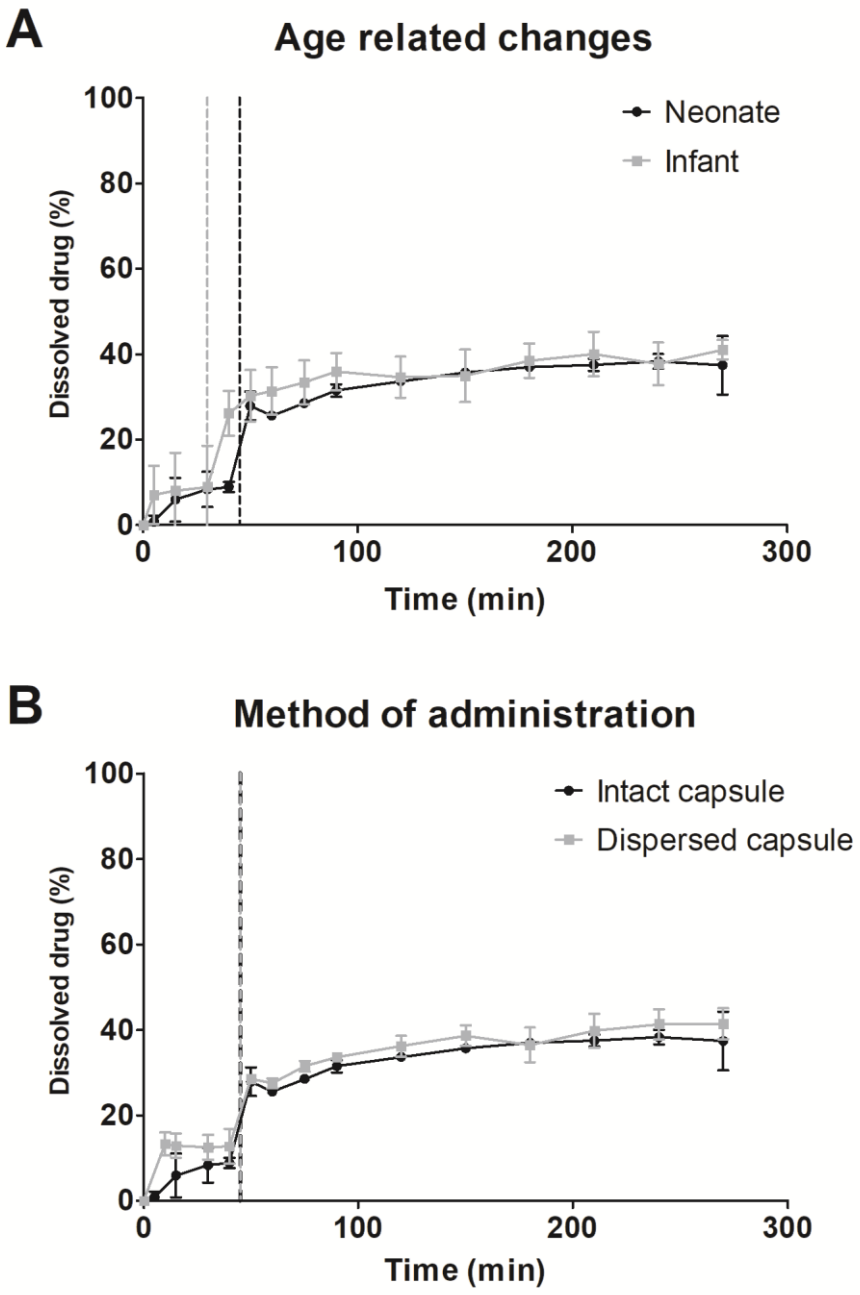

B
Formulation
Tablet
Capsule
Dissolved drug (%)
100
80
60
40
20
0
0
100
200
300
Time (min)


C
USP II mini vessel vs. USP IV
USP II
USP IV
Dissolved drug (%)
100
80
60
40
20
0
0
100
200
300
Time (min)

587

588

589



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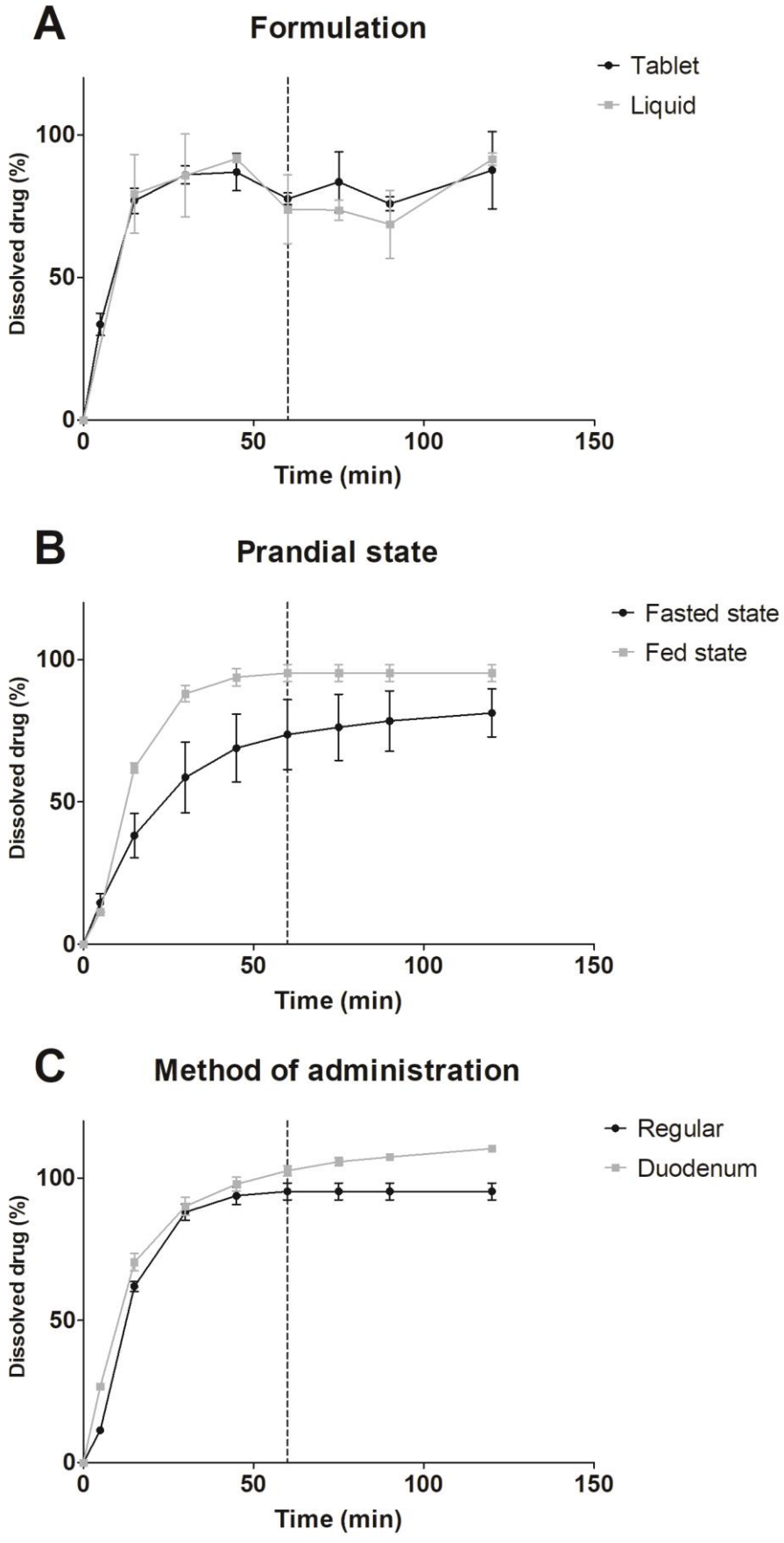
A
Formulation
Tablet
Liquid
Dissolved drug (%)
100
50
0
0
50
100
150
Time (min)
B
Prandial state
Fasted state
Fed state
Dissolved drug (%)
100
50
0
0
50
100
150
Time (min)
C
Method of administration
Regular
Duodenum
Dissolved drug (%)
100
50
0
0
50
100
150
Time (min)

593